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PREGNANCY INTERFERING ACTION OF LHRH AND ANTI-LHRH

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Summary—Until recently LHRH was believed to be a product of the hypothalamic origin whose primary function was to regulate the secretion of gonadotropin from the pituitary. In the last few years, a large body of experimental evidence has emerged for the existence of the releasing hormone at extra-hypothalamic sites. The placenta is one such organ in which the hormone is made and probably has a role in stimulation of the secretion of chorionic gonadotropin as suggested by both *in vivo* and *in vitro* experiments. Superagonists of LHRH as well as monoclonal antibodies raised against the decapeptide administered during early pregnancy bring about a sharp decrease in blood chorionic gonadotropin and progesterone levels followed by termination of pregnancy in baboons. The mechanism of the abortifacient action appears to be curtailment of chorionic gonadotropin secretion by the placenta. Whether the immunointerception through LHRH andibodies will hold true for human beings also has to be investigated.

INTRODUCTION

The potential of the approach to the control of fertility and infertility with the simple decapeptide hypophysiotropic hormone LHRH is much greater than was initially envisaged. Moderate carefully timed doses of LHRH or its agonists induce ovulation but by repeated administration paradoxical antifertility effects are exerted [1, 2]. The suppression of gonadal function during long-term treatment with the LHRH analogues is ascribable to inhibition of gonadotropin secretion caused by the down grading action of the decapeptide at the pituitary level [3, 4]. A direct gonadal site of action of LHRH resulting in decreased normal physiological functions of these organs has also been observed [5]. LHRH or its agonistic analogues administered in the early or mid-luteal phase of the menstrual cycle have been found to induce short-luteal phase as indicated by reduced progesterone levels in serum and early onset of menstruation [6, 7]. It was suggested that the luteolytic activity was due to "down-regulation" of LH receptors in the ovary mediated by the increase of endogenous LH or caused by a direct inhibitory effect of the LHRH agonist on target cells in the ovary.

We have conducted experiments in baboons to test whether administration of LHRH agonist or injection of bioneutralizing monoclonal antibodies against the native decapeptide during the luteal phase can suppress progesterone secretion to an extent that can result in termination of pregnancy in these animals.

EFFECT OF LHRH AGONIST ADMINISTRATION AROUND PERIIMPLANTATION PERIOD

We have earlier shown that the LHRH agonist D-Ser (Bu^t)^6 des Gly^{10}Pro EA (Hoe 766) at a dose of 50 μg twice daily for a minimum period of 4 days given subcutaneously to pregnant baboons successfully terminated pregnancy in all the animals tested [8]. The timing of the treatment is important as even more frequent administration (thrice daily) of the agonist given at early luteal phase though successful in suppressing luteal function considerably, failed to abrogate pregnancy. Positive results were obtained only when the agonist was given towards the end of the luteal phase around the time when chorionic gonadotropin was detectable in circulation.

EFFECT OF ANTI-LHRH ANTIBODIES ON PREGNANCY

Studies were carried out in 5 baboons in which instead of repeated administration of LHRH agonist at the peri-implantation period, two injections of monoclonal antibodies of 2 ml each were given at 24 h interval, on the grounds that the rise in chorionic gonadotropin is steep in early pregnancy and the hormonal support has to be nullified for at least 48 h to influence pregnancy. In three out of five baboons tested, this amount of anti-LHRH monoclonals brought about termination of pregnancy (Fig. 1). Soon after the administration of anti-LHRH antibodies a drop in the chorionic gonadotropin level was perceptible which was accompanied by a decrease in progesterone. Out of the two baboons which failed to abort after antibody administration, one animal aborted on 50th day of LMP (Fig. 2). In this baboon, plasma progesterone concentration remained low and there was no rise in plasma steroid level characteristic of pregnancy. The failure of rise in circulating levels of progesterone despite increasing levels of chorionic gonadotropin in this animal is similar to the findings with LHRH agonist in baboons and suggest a direct

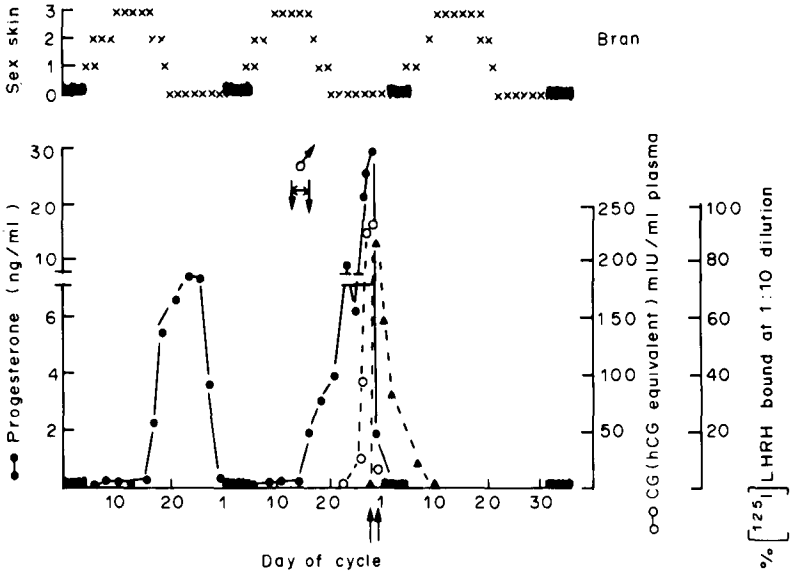

Fig. 1. Termination of pregnancy in a baboon by anti-LHRH monoclonals. Two injections of 2 ml ascites fluid were given intravenously on days 28 and 29 of the cycle. Antibody caused a steep decline in CG and progesterone levels followed by bleeding (black rectangles). Circulating antibody levels measured by RIA are represented by triangles. Progesterone profile of the premating cycle is also given.

gonadal effect of these agents [9]. The second animal which failed to abort after two consecutive injections of the antibodies was given another injection of 5 ml ascitic fluid 4 days later (Fig. 3). At this time chorionic gonadotropin levels had started rising and higher amounts of antibodies were ineffective in suppressing either chorionic gonadotropin or progesterone secretion. Pregnancy in this animal continued uneventfully to term. On careful analysis, the CG levels in this baboon were found to be much higher than the three other animals in which pregnancy was terminated. After regaining her menstrual cycles, the baboon was rendered pregnant again and this time 5 ml ascites fluid was given on two subsequent days (total of 10 ml) following the same protocol as that of previous experiment (Fig. 4). A sharp fall in circulating progesterone and chorionic gonadotropin level was observed after the administration of antibodies and the animal aborted 2 days later.

To rule out any non-specific effect of the ascites fluid on pregnancy, product of a non-secretory clone was injected in another pregnant baboon following a schedule parallel to that of earlier experiment (Fig. 5). Pregnancy in this animal was carried through term uninterrupted.

The mechanisms by which LHRH antibodies act in

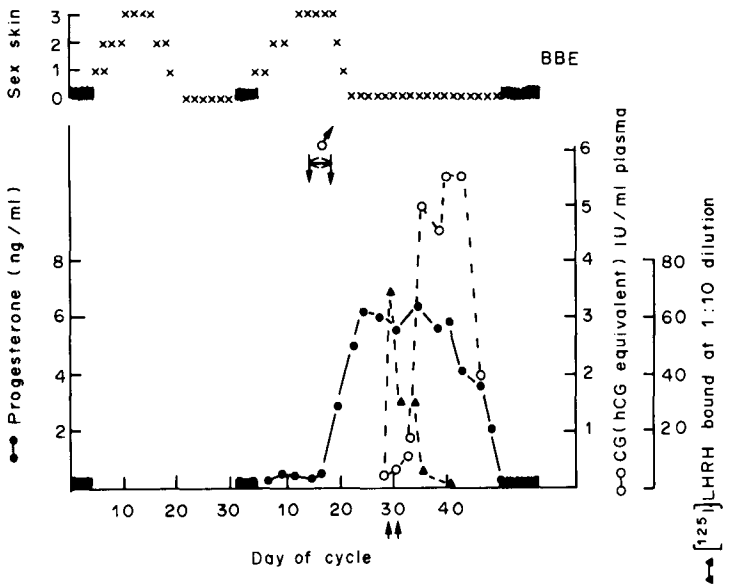

Fig. 2. Plasma progesterone and chorionic gonadotropin levels in a baboon injected with anti-LHRH monoclonals in which abortion took place on the 50th day after LMP. Progesterone level does not show any rise in presence of circulating chorionic gonadotropin.

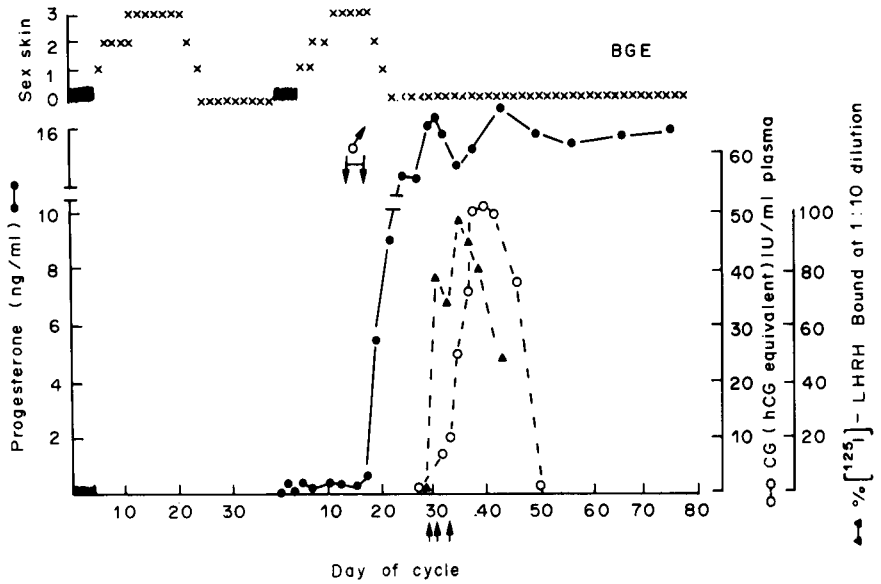

Fig. 3. Failure of anti-LHRH monoclonals to terminate pregnancy in a baboon. The animal failed to abort after two consecutive injections of the antibodies. A third injection of 5 ml ascites given 3 days later was also ineffective.

termination of pregnancy can be either by luteolytic action or by interference with placental functions. A drop in chorionic gonadotropin by anti-LHRH antibodies indicate an apparent relationship between the two hormones.

Human placenta has been shown to synthesize and contain large quantities of biologically active LHRH which is biochemically and immunologically indistinguishable from hypothalamic LHRH [10]. Also human placental tissue is able to incorporate radioactive amino acids into a material having chromatographic, immunologic and biologic activity identical to hypothalamic LHRH [11]. Using immunofluorescent probes, LHRH was shown to be localized in the cytotrophoblast, syncytiotrophoblast and in the villous stroma [12]. Studies of Currie *et al.* [13] show that there are specific receptors for LHRH in human placenta. Using human placenta in culture Khodr and Siler-Khoder [14] have demonstrated a specific dose dependent stimulation of hCG secretion by LHRH. An increase in circulating chorionic gonadotropin levels by LHRH has also been observed in pregnant bonnet monkeys [15]. These data indicate that the primate placenta not only contains LHRH but is also responsive to the stimulation of LHRH. The decapeptide seems to regulate the production of

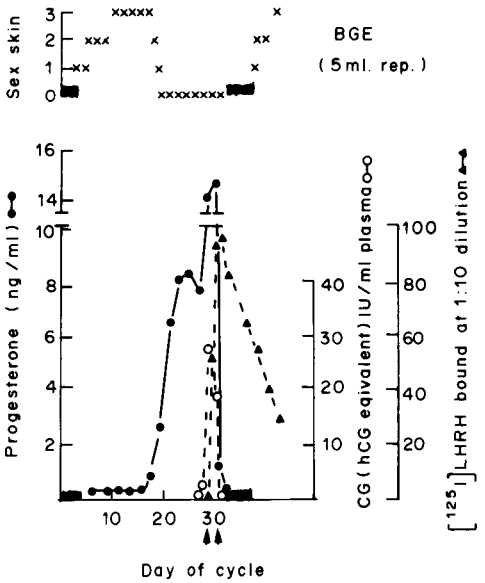

Fig. 4. Two injections of 5 ml of ascites fluid (total 10 ml) given intravenously on the day when chorionic gonadotropin was measurable to the same baboon BGE of Fig. 3 in a subsequent pregnancy: Antibody caused a steep decline in CG and progesterone levels followed by menstrual bleeding.

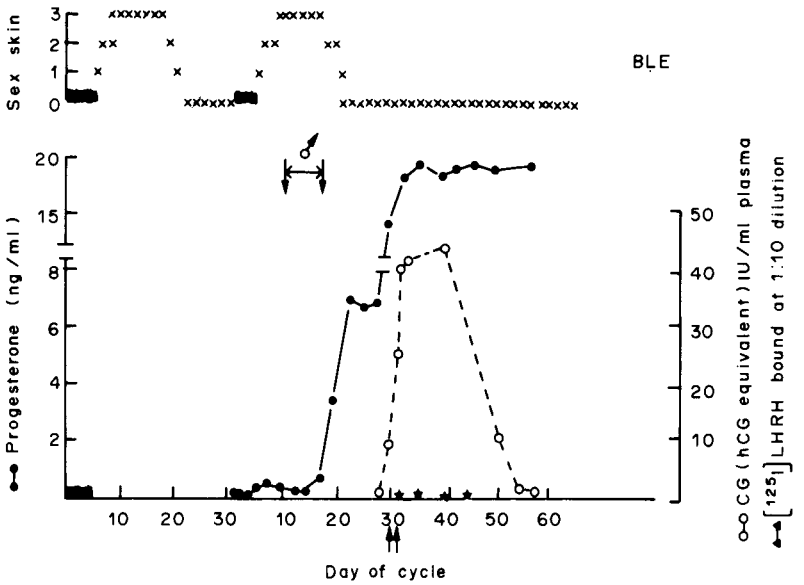

Fig. 5. Effect of injection of a non-secretory clone product to a pregnant baboon. Plasma chorionic gonadotropin and progesterone remain unaffected.

chorionic gonadotropin and possibly anti-LHRH antibodies intercept this action to bring about termination of pregnancy.

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